

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

Claims 1 to 357 (Canceled).

358. (New) A method for delivering a polypeptide into a vertebrate, comprising administering into a tissue or cavity of said vertebrate a composition comprising:

- (a) about 1 ng to about 30 mg of a polynucleotide in aqueous solution which operably encodes a polypeptide upon delivery to vertebrate cells *in vivo*;
- (b) a salt M-X dissolved in said aqueous solution at a molar concentration ranging from about 50 mM to about 250 mM, and reaction, association, and dissociation products thereof, wherein M is a cation selected from the group consisting of sodium and potassium, wherein X is an anion selected from the group consisting of phosphate, acetate, bicarbonate, sulfate, and pyruvate; and
- (c) an auxiliary agent selected from the group consisting of a poloxamer and a reverse poloxamer;

wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 50 mM, and wherein said polypeptide is expressed in the vertebrate in an amount sufficient to be detectable.

359. (New) The method of claim 358, wherein the auxiliary agent is a poloxamer.

360. (New) The method of claim 358, wherein the auxiliary agent is a reverse poloxamer.

361. (New) The method of claim 359, wherein the poloxamer has a molecular weight from 1000 to greater than 16000.

362. (New) The method of claim 359, wherein the poloxamer has an approximate hydrophobe molecular weight from 900 to 3600 and an approximate hydrophile weight percentage of 10% to 80%.

363. (New) The method of claim 360, wherein the reverse poloxamer has an approximate hydrophobe molecular weight of 1000 to 3100 and an approximate hydrophile weight percentage of 10% to 80%.

364. (New) The method of claim 363, wherein the reverse poloxamer has an approximate hydrophobe molecular weight of about 2500 and an approximate hydrophile weight percentage of about 20%.

365. (New) The method of claim 359, wherein the poloxamer is selected from the group consisting of a poloxamer having an approximate hydrophobe molecular weight

of 1800 and an approximate hydrophile weight percentage of 80%; a poloxamer having an approximate hydrophobe molecular weight of 2100 and an approximate hydrophile weight percentage of 70%; a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 80%; a poloxamer having an approximate hydrophobe molecular weight of 2400 and an approximate hydrophile weight percentage of 40%; a poloxamer having an approximate hydrophobe molecular weight of 1200 and an approximate hydrophile weight percentage of 40%; a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 20%; and a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 40%.

366. (New) The method of claim 360, wherein the reverse poloxamer is selected from the group consisting of a reverse poloxamer having an approximate hydrophobe molecular weight of 1700 and an approximate hydrophile weight percentage of 40%; a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 40%; and a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

367. (New) The method of claim 366, wherein the reverse poloxamer has an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

368. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of about 0.1% (w/v) to about 6.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 80%; about 0.001% (w/v) to about 2.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 2100 and an approximate hydrophile weight percentage of 70%; and about 0.01% (w/v) to about 1% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 50%.

369. (New) The method of claim 360, wherein the reverse poloxamer has an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20% and is present in the composition in a concentration of about 0.001% (w/v) to about 1.0% (w/v).

370. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of about 0.5% to about 4.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 80%; about 0.1% (w/v) to about 1.7% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 2100 and an approximate hydrophile weight percentage of 70%; and about 0.01% (w/v) to about 0.5% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 40%.

371. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of 4% a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 80%; 1.0% (w/v) a poloxamer having an approximate hydrophobe molecular weight of 2100 and an approximate hydrophile weight percentage of 70%; and 0.5% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 50%.

372. (New) The method of claim 359, wherein the poloxamer is selected from the group consisting of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 50%; a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 30%; a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 40%; a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 50%; a poloxamer having an approximate hydrophobe molecular weight of 3600 and an approximate hydrophile weight percentage of 30%; a poloxamer having an approximate hydrophobe molecular weight of 900 and an approximate hydrophile weight percentage of 10%; a poloxamer having an approximate hydrophobe molecular weight of 1200 and an approximate hydrophile weight percentage of 30%; a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 10%; a poloxamer having an approximate hydrophobe molecular weight of 2400 and an approximate hydrophile weight percentage of 10%; a poloxamer having an

approximate hydrophobe molecular weight of 2700 and an approximate hydrophile weight percentage of 20%; a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 10%; and a poloxamer having an approximate hydrophobe molecular weight of 3600 and an approximate hydrophile weight percentage of 10%.

373. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of about 0.01% (w/v) to about 1.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 80%; about 0.01% (w/v) to about 1.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 30%; about 0.0005% (w/v) to about 1.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1200 and an approximate hydrophile weight percentage of 40%; about 0.01% (w/v) to about 1.0% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 40%; about 0.002% (w/v) to about 1.0% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 1700 and an approximate hydrophile weight percentage of 40%; about 0.002% (w/v) to about 1.0% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 40%; and about 0.001% (w/v) to about 1.0% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

374. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of about 0.05% (w/v) to about 0.5% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 80%; about 0.1% (w/v) to about 1% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 50%; about 0.05% (w/v) to about 0.10% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 30%; about 0.001% (w/v) to about 0.10% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1200 and an approximate hydrophile weight percentage of 40%; about 0.01% (w/v) to about 0.10% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 1700 and an approximate hydrophile weight percentage of 40%; about 0.01% (w/v) to about 0.10% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 40%; and about 0.001% (w/v) to about 0.1% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

375. (New) The method of claim 374, wherein the auxiliary agent is about 0.001% (w/v) to about 0.1% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

376. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of 0.1% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 80%; 0.05% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 3000 and an approximate hydrophile weight percentage of 30%; 0.001% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1200 and an approximate hydrophile weight percentage of 40%; about 0.01% (w/v) to about 0.1% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 40%; 0.10% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 1700 and an approximate hydrophile weight percentage of 40%; 0.01% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 40%; and 0.01% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

377. (New) The method of claim 376, wherein the auxiliary agent is 0.01% (w/v) of a reverse poloxamer having an approximate hydrophobe molecular weight of 2500 and an approximate hydrophile weight percentage of 20%.

378. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of about 0.001% (w/v) to about 0.1% (w/v) a poloxamer having an approximate hydrophobe molecular weight of 900 and an approximate hydrophile weight percentage of 10%; about 0.001% (w/v) to about 0.1% (w/v) a poloxamer having an

approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 10%; and about 0.001 % (w/v) to about 1.0% (w/v) a poloxamer having an approximate hydrophobe molecular weight of 2700 and an approximate hydrophile weight percentage of 20%.

379. (New) The method of claim 358, wherein the auxiliary agent is selected from the group consisting of 0.05% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 900 and an approximate hydrophile weight percentage of 10%; 0.01% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 1800 and an approximate hydrophile weight percentage of 10%; and 0.05% (w/v) of a poloxamer having an approximate hydrophobe molecular weight of 2700 and an approximate hydrophile weight percentage of 20%.

380. (New) The method of claim 358, wherein M is selected from the group consisting of sodium and potassium, and wherein X is selected from the group consisting of phosphate, acetate, and bicarbonate.

381. (New) The method of claim 380, wherein said salt is sodium phosphate or potassium phosphate.

382. (New) The method of claim 358, wherein said salt is dissolved in said aqueous solution at a concentration ranging from about 100 mM to 200 mM.

383. (New) The method of claim 358, wherein said salt is dissolved in said aqueous solution at a concentration of about 150 mM.

384. (New) The method of claim 358, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 30 mM.

385. (New) The method of claim 358, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 15 mM.

386. (New) The method of claim 358, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 5 mM.

387. (New) The method of claim 358, wherein said polynucleotide is DNA operably associated with a promoter.

388. (New) The method of claim 387, wherein said polynucleotide is contained on a plasmid.

389. (New) The method of claim 358, wherein said polynucleotide is RNA.

390. (New) The method of claim 389, wherein said polynucleotide is contained in messenger RNA.

391. (New) The method of claim 358, wherein said polypeptide is selected from the group consisting of a therapeutic polypeptide, an antigenic polypeptide, an immunogenic polypeptide, an immunomodulatory polypeptide, and a functional self polypeptide.

392. (New) The method of claim 391, wherein said therapeutic polypeptide is selected from the group consisting of granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor colony stimulating factor, interleukin 2, interleukin-3, interleukin 4, interleukin 5, interleukin 6, interleukin 7, interleukin 8, interleukin 10, interleukin 12, interleukin 15, interleukin 18, interferon alpha, interferon beta, interferon gamma, interferon omega, interferon tau, interferon gamma inducing factor I, transforming growth factor beta, RANTES, macrophage inflammatory proteins, Leishmania elongation initiating factor, platelet derived growth factor, tumor necrosis factor, epidermal growth factor, vascular epithelial growth factor, fibroblast growth factor, nerve growth factor, brain derived neurotrophic factor, neurotrophin-2, neurotrophin-3, neurotrophin-4, neurotrophin-5, glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, erythropoietin, insulin, and therapeutically active fragments, analogs, or derivatives thereof.

393. (New) The method of claim 391, wherein said immunogenic polypeptide is selected from the group consisting of a bacterial polypeptide, a viral polypeptide, a fungal polypeptide, a parasite polypeptide, an allergen, a tumor specific polypeptide, and immunogenic fragments, analogs, or derivatives thereof.

394. (New) The method of claim 391, wherein said immunomodulatory polypeptide is selected from the group consisting of a cytokine, a chemokine, and immunomodulatory fragments, analogs, or derivatives thereof.

395. (New) The method of claim 391, wherein said functional self polypeptide is selected from the group consisting of insulin, dystrophin, cystic fibrosis transmembrane conductance regulator, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor colony stimulating factor, interleukin 2, interleukin-3, interleukin 4, interleukin 5, interleukin 6, interleukin 7, interleukin 8, interleukin 10, interleukin 12, interleukin 15, interleukin 18, interferon alpha, interferon beta, interferon gamma, interferon omega, interferon tau, interferon gamma inducing factor I, transforming growth factor beta, RANTES, macrophage inflammatory proteins, platelet derived growth factor, tumor necrosis factor, epidermal growth factor, vascular epithelial growth factor, fibroblast growth factor, nerve growth factor, brain derived neurotrophic factor, neurotrophin-2, neurotrophin-3, neurotrophin-4, neurotrophin-5, glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, erythropoietin, and therapeutically active fragments, analogs, and derivatives thereof.

396. (New) The method of claim 358, further comprising a transfection facilitating agent selected from the group consisting of cationic lipids, calcium phosphate, alum, gold, tungsten, or other metal particles, transfection facilitating peptides, transfection facilitating proteins, and transfection facilitating polymers.

397. (New) The method of claim 396, wherein said transfection facilitating agent is a cationic lipid.

398. (New) The method of claim 358, wherein said vertebrate is a mammal.

399. (New) The method of claim 398, wherein said mammal is a human.

400. (New) The method of claim 358, wherein said tissue is selected from the group consisting of muscle, skin, brain tissue, lung tissue, liver tissue, spleen tissue, bone marrow tissue, thymus tissue, heart tissue, lymph tissue, blood tissue, bone tissue, connective tissue, mucosal tissue, pancreas tissue, kidney tissue, gall bladder tissue, intestinal tissue, testicular tissue, ovarian tissue, uterine tissue, vaginal tissue, rectal tissue, nervous system tissue, eye tissue, glandular tissue, and tongue tissue.

401. (New) The method of claim 358, wherein said cavity is selected from the group consisting of the lungs, the mouth, the nasal cavity, the stomach, the peritoneal cavity, the intestine, a heart chamber, veins, arteries, capillaries, lymphatic cavities, the uterine cavity, the vaginal cavity, the rectal cavity, joint cavities, ventricles in brain, spinal canal in spinal cord, and the ocular cavities.

402. (New) The method of claim 358, wherein said cavity comprises a mucosal surface.

403. (New) The method of claim 402 wherein said mucosal surface is lung tissue.

404. (New) The method of claim 400, wherein said tissue is muscle.

405. (New) The method of claim 404, wherein said tissue is skeletal muscle, smooth muscle, or myocardium.

406. (New) The method of claim 358, wherein said administration is by a route selected from the group consisting of intramuscular, intravenous, intratracheal, intranasal, transdermal, interdermal, subcutaneous, intraocular, vaginal, rectal, intraperitoneal, intrainestinal and inhalation.

407. (New) The method of claim 358, wherein said administration route is intravenous.

408. (New) The method of claim 358, wherein said administration route is intramuscular.

409. (New) The method of claim 408, wherein said administration is by intramuscular injection.

410. (New) The method of claim 358, wherein said administration is mediated by a catheter.

411. (New) A composition for delivering a polypeptide into a vertebrate, comprising administering into a tissue or cavity of said vertebrate a composition comprising:

(a) about 1 ng to about 30 mg of a polynucleotide in aqueous solution which operably encodes a polypeptide upon delivery to vertebrate cells *in vivo*;

(b) a salt M-X dissolved in said aqueous solution at a molar concentration ranging from about 50 mM to about 250 mM, and reaction, association, and dissociation products thereof, wherein M is a cation selected from the group consisting of sodium and potassium, wherein X is an anion selected from the group consisting of phosphate, acetate, sulfate, and pyruvate; and

(c) an auxiliary agent selected from the group consisting of a poloxamer and a reverse poloxamer;

wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 50 mM, and wherein said polypeptide is expressed in the vertebrate in an amount sufficient to be detectable.

412. (New) The composition of claim 411, wherein said salt is dissolved in said aqueous solution at a concentration ranging from about 100 mM to 200 mM.

413. (New) The composition of claim 411, wherein said salt is dissolved in said aqueous solution at a concentration of about 150 mM.

414. (New) The composition of claim 411, wherein X of said salt is phosphate.

415. (New) The composition of claim 411, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 30 mM.

416. (New) The composition of claim 411, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 15 mM.

417. (New) The composition of claim 411, wherein said aqueous solution contains chloride ion at a molar concentration ranging from 0 mM to about 5 mM.